CLAIMS

It is claimed:

1. A method for determining the relative abundance of a nucleic acid sequence among at least two nucleic acid populations, comprising:

(a) contacting with a reference library which comprises multiple copies of a selected nucleic acid sequence:

a first probe, derived from a first nucleic acid population, having a sequence which is complementary to said selected sequence and a terminal first sample ID (SID) sequence, and

a second probe, derived from a second nucleic acid population, having a sequence which is complementary to said selected sequence and a terminal second sample ID (SID) sequence;

wherein said first and second probes are present in relative amounts proportional to the abundance of the nucleic acid sequence in the respective populations,

whereby, upon said contacting,

(i) said first and second probes competitively hybridize with said selected sequence in said reference library, such that said probes are present in duplexes in relative amounts proportional to the abundance of the nucleic acid sequence in the respective populations, and said SID sequences are present as single stranded extensions on said duplexes; and

(ii) said first SID sequences on said duplexes and said second SID sequences on said duplexes hybridize with each other in a 1:1 ratio;

and

(b) detecting the presence of unhybridized SID sequences, as an indication of the relative amounts of hybridized first probe and hybridized second probe.

2. The method of claim 1, wherein in said contacting step (a), a plurality of probes derived from said first population and a plurality of probes derived from said second population are contacted with said reference library, and

said reference library comprises multiple copies of the sequences present in the first and second populations, such that different sequences within the library are attached to spatially distinct solid phase supports in clonal subpopulations.

3. The method of claim 2, wherein said spatially distinct solid phase supports are separate regions of a planar support.

4. The method of claim 2, wherein said spatially distinct solid phase supports are microparticles.

5. The method of claim 2, wherein said detecting comprises attaching to each said unhybridized SID sequence a labeled decoder moiety.

6. The method of claim 5, wherein a first light-generating label is present on first decoder moieties selectively attachable to unhybridized first SID sequences, and a second, distinguishable light generating label is present on second decoder moieties selectively attachable to unhybridized second SID sequences.

7. The method of claim 6, wherein each said decoder moiety includes a terminal oligonucleotide sequence that is complementary to either said first or said second SID sequence.

8. The method of claim 5, wherein said decoder labels are fluorescent dye molecules.

9. The method of claim 8, wherein said each said decoder label comprises multiple fluorescent dye molecules.

10. The method of claim 8, wherein said spatially distinct solid phase supports are microparticles, and further comprising:

sorting said microparticles by FACS according to the ratio of fluorescent signals generated by said fluorescent labels on each microparticle.

11. The method of claim 10, further comprising:

accumulating in a separate vessel each said microparticle having a value of said ratio of fluorescent signals within one or more selected ranges of values; and

determining a nucleotide sequence of a portion of the nucleic acid sequence on one or more of said microparticles.

12. The method of claim 6, wherein said first and second probes are further labeled with said first and second light-generating labels, respectively.

13. The method of claim 6, wherein a known fraction of said first and second probes are further labeled with said first and second light-generating labels, respectively.

14. The method of claim 1, wherein said first and second SID sequences are complementary and thus hybridize with each other directly.

15. The method of claim 1, wherein said first and second SID sequences hybridize with each other through an intermediate molecule comprising sequences complementary to said first and second SID sequences, and wherein the method further comprises, concurrent with or following step (a), contacting said intermediate molecule with said reference library and first and second probes.

16. The method of claim 2, for use in analysis of differentially regulated or expressed genes, wherein said populations are cDNA libraries derived from expressed genes of each of a plurality of sources selected from different cells, tissues, or individuals; and said reference DNA library is derived from genes expressed in the plurality of different sources.

17. The method of claim 2, for use in analysis of genetic variations among individuals or populations of individuals, wherein said populations are genomic DNA libraries derived from different individuals or populations of individuals; and said reference DNA library is derived from pooled genomic DNA of such individuals or populations of individuals.

18. A kit for use in determining the relative abundance of nucleic acid sequences among at least two nucleic acid populations derived from a plurality of sources selected from different cells, different tissues, different individuals, and different populations of

individuals; the kit comprising:

(i) a reference nucleic acid library containing the sequences present in the plurality of different sources, wherein different sequences within the library are attached to separate solid phase supports in clonal subpopulations;

(ii) a first plurality of probes, derived from a nucleic acid library from one of said plurality of sources, each probe having appended a terminal first SID sequence, and

(iii) a second plurality of probes, derived from a nucleic acid library from a second of said plurality of sources, each probe having appended a terminal second SID sequence, which is able to hybridize with said first SID sequence.

19. The kit of claim 18, further comprising

(iv) a first decoder moiety, selectively attachable to said first SID sequence, having a first light-generating label, and

(v) a second decoder moiety, selectively attachable to said second SID sequence, having a second, distinguishable light-generating label.

20. The kit of claim 19, wherein each said decoder moiety is an oligonucleotide having a terminal sequence complementary to said first or second SID sequence, respectively, and each said label comprises a fluorescent dye molecule.

21. The kit of claim 20, wherein each said decoder moiety comprises multiple fluorescent dye molecules.

22. The kit of claim 19, wherein said first and second pluralities of probes are further labeled with said first and second distinguishable light-generating labels, respectively.

23. The kit of claim 19, wherein a known fraction of said first and second pluralities of probes are further labeled with said first and second distinguishable light-generating labels, respectively.

24. A kit for use in preparation of sequence ID (SID) tagged probes, competitive hybridization, and SRQ decoding, for use in determining the relative abundance of

nucleic acid sequences among two or more nucleic acid populations; the kit comprising:

(a) two or more SID adaptors for generating SID tagged probes, each said adaptor comprising a double stranded oligonucleotide having, in sequence: (i) a protruding single strand effective for ligation to a DNA restriction fragment, (ii) a sample ID (SID) sequence, (ii) a restriction site, and (iv) a primer binding site,

wherein cleavage by an enzyme recognizing said restriction site is effective to cleave all but elements (i) and (ii) from said adaptor, and wherein different adaptors have different sample ID (SID) sequences which are able to hybridize with each other; and

(b) two or more sample ID (SID) decoders, selectively attachable to said different SID sequences, and having distinguishable light-generating labels.

25. The kit of claim 24, further comprising a reference nucleic acid library containing DNA sequences present in the two or more nucleic acid populations, wherein different sequences within the library are attached to separate solid phase supports in clonal subpopulations.

26. A method for sorting a population of nucleic acid sequences in accordance with their relative abundance between at least two nucleic acid populations, comprising:

(a) contacting with a reference library which comprises the nucleic acid sequences present in the nucleic acid populations, such that different sequences within the library are attached to spatially distinct solid phase supports in clonal subpopulations:

a plurality of probes derived from said first population, each probe having a sequence which is complementary to a reference library sequence, and a terminal first sample ID (SID) sequence, and

a plurality of probes derived from said second population, each probe having a sequence which is complementary to a reference library sequence, and a terminal second sample ID (SID) sequence,

wherein said first and second probes having a given sequence, exclusive of the SID sequence, are present in relative amounts proportional to the abundance of said sequence in the respective populations,

and whereby, upon said contacting,

(i) said first and second probes competitively hybridize with complementary

sequences in said reference library, such that said probes of a given sequence are present in duplexes in relative amounts proportional to the abundance of that nucleic acid sequence in the respective populations, and said SID sequences are present as single stranded extensions on said duplexes; and

(ii) said first SID sequences on said duplexes and said second SID sequences on said duplexes hybridize with each other in a 1:1 ratio;

(b) applying to each unhybridized SID sequence a decoder moiety having a fluorescent label, wherein a first fluorescent label is present on first decoder moieties selectively attachable to unhybridized first SID sequences, and a second, distinguishable fluorescent label is present on second decoder moieties selectively attachable to unhybridized second SID sequences; and

(c) sorting said microparticles by FACS according to the ratio of fluorescent signals generated by said fluorescent labels on each microparticle.

27. The method of claim 26, wherein each said decoder moiety includes a terminal oligonucleotide sequence that is complementary to either said first or said second SID sequence.

28. The method of claim 27, wherein said each said decoder moiety comprises multiple fluorescent molecules.